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## 5 WHAT IS CLAIMED IS:

1. A system comprising:

- (a) a light source for illuminating a tissue sample;
- (b) a detector for detecting light transmitted through the sample; and
- (c) a processor for calculating spatial second derivatives of products of sample

10 thicknesses and intensities of the transmitted light.

2. The system of claim 1, wherein the light source comprises a laser diode.

3. The system of claim 1, wherein the light source illuminates the sample with light having at least two wavelengths.

4. The system of claim 1, wherein the light source illuminates the sample with light  
15 having at least one wavelength in the near infrared spectrum.

5. The system of claim 1, wherein the detector comprises a photomultiplier tube or an avalanche photodiode.

6. The system of claim 1, wherein the processor is a computer.

7. The system of claim 1, wherein the spatial second derivatives,  $N''(x,y)$ , are calculated  
20 from an equation  $N(x,y) = r_0 ac_0 / r(x,y) ac(x,y)$ , wherein

$x$  and  $y$  are respective x- and y-coordinates of the coplanar locations;

$r_0$  is a maximum thickness of the sample;

$ac_0$  is the intensity at the location where the thickness of the sample is  $r_0$ ;

$r(x,y)$  is the thickness of the sample at location  $(x,y)$ ;

25  $ac(x,y)$  is the intensity at location  $(x,y)$ ; and

$N(x,y)$  is dimensionless number at a location  $(x,y)$ .

- 5 8. The system of claim 1, further comprising (d) a monitor to display an image of the spatial second derivatives.
9. The system of claim 1, further comprising (d) a computer-readable medium to store values of the spatial second derivatives.
10. A computer-readable medium comprising computer-readable instructions for causing  
10 a computer system to:
- (a) receive data from a tissue sample that represent measures of thicknesses of the sample and intensities of light transmitted through the sample; and
  - (b) calculate spatial second derivatives of products of the thicknesses of the sample and intensities of the transmitted light to generate values that represent an image  
15 of the sample.
11. The computer-readable medium of claim 10, further comprising computer-readable instructions for causing the computer system calculate a spatial second derivative from an equation  $N(x,y) = r_0 ac_0 / r(x,y) ac(x,y)$  to generate values,  $N''(x,y)$ , wherein
- $x$  and  $y$  are respective x- and y-coordinates of the values;
  - 20  $r_0$  is a maximum thickness of the sample;
  - $ac_0$  is the intensity at a location where the thickness of the sample is  $r_0$ ;
  - $r(x,y)$  is the thickness of the sample at location  $(x,y)$ ; and
  - $ac(x,y)$  is the intensity at location  $(x,y)$ .

5 12. A computer-readable medium comprising computer-readable instructions for causing a computer system to:

(a) receive data from a sample representing measures of intensities of light transmitted through the sample for at least two wavelengths of light;

(b) select a pair of wavelengths to minimize a difference between relative changes in the intensities of the light transmitted through a region of the sample, wherein the relative changes in intensities of the light are measured relative to a background intensity of light transmitted through the sample; and

(c) process the relative changes in intensities of the light at the pair of wavelengths using measures of a background reduced scattering coefficient of the sample at the pair of wavelengths and molar extinction coefficients for deoxy-hemoglobin and oxy-hemoglobin to generate data values represent an absolute level of oxygenation of the region of the sample.

13. The computer-readable medium of claim 12, further comprising computer-readable instructions for causing the computer system to generates values of the absolute level of oxygenation,  $SO_2$ , of the region of the sample using a formula

$$SO_2 = \frac{\epsilon_{Hb}(\lambda_2) - \epsilon_{Hb}(\lambda_1) \frac{\mu'_{s0}(\lambda_1)}{\mu'_{s0}(\lambda_2)} \frac{\Delta I / I_0|_{\max}^{(\lambda_2)}}{\Delta I / I_0|_{\max}^{(\lambda_1)}}}{[\epsilon_{Hb}(\lambda_2) - \epsilon_{HbO_2}(\lambda_2)] + [\epsilon_{HbO_2}(\lambda_1) - \epsilon_{Hb}(\lambda_1)] \frac{\mu'_{s0}(\lambda_1)}{\mu'_{s0}(\lambda_2)} \frac{\Delta I / I_0|_{\max}^{(\lambda_2)}}{\Delta I / I_0|_{\max}^{(\lambda_1)}}},$$

wherein:

$\Delta I / I_0|_{\max}^{(\lambda_1)}$  is a maximum value of a relative change in intensity at a first wavelength in the pair of wavelengths;

$\Delta I / I_0|_{\max}^{(\lambda_2)}$  is a maximum value of a relative change in intensity at a second wavelength in the pair of wavelengths;

5  $\frac{\mu'_{s0}(\lambda_1)}{\mu'_{s0}(\lambda_2)}$  is a ratio of the background reduced scattering coefficient at the first wavelength to the background reduced scattering coefficient at the second wavelength;  
 $\epsilon_{Hb}(\lambda_1)$  and  $\epsilon_{Hb}(\lambda_2)$  are the molar extinction coefficients for deoxy-hemoglobin at the first and second wavelengths; and  
 $\epsilon_{HbO2}(\lambda_1)$  and  $\epsilon_{HbO2}(\lambda_2)$  are the molar extinction coefficients for oxy-hemoglobin  
 10 at the first and second wavelengths.

14. A method of detecting a region of a tissue sample having an optical absorption level different from a background optical absorption level, the method comprising:  
 (a) obtaining thicknesses of the sample and intensities of light transmitted through  
 15 the sample at a plurality of locations;  
 (b) calculating spatial second derivatives of products of the sample thicknesses and the intensities of the transmitted light at the locations; and  
 (c) comparing the spatial second derivatives to detect a region of the sample having an optical absorption level different from a background level of optical absorption  
 20 in the sample.

15. The method of claim 14, where the tissue sample is selected from the group consisting of breast tissue, brain tissue, and muscle tissue.

16. The method of claim 14, wherein the optical absorption is a characteristic of absorption of light by hemoglobin in the sample.

25 17. The method of claim 14, wherein the region of the sample having an optical absorption level different from the background level of optical absorption within the sample comprises a lesion, tumor, or cancerous tissue.

18. The method of claim 14, wherein the transmitted light is generated by coplanar sources and wherein the plurality of locations are coplanar.

- 5 19. The method of claim 18, wherein the spatial second derivatives,  $N''(x,y)$ , are calculated from an equation  $N(x,y) = r_0 ac_0 / r(x,y) ac(x,y)$ , wherein
- $x$  and  $y$  are respective x- and y-coordinates of the coplanar locations;  
 $r_0$  is a maximum thickness of the sample;  
 $ac_0$  is an intensity at the location where the thickness of the sample is  $r_0$ ;  
10  $r(x,y)$  is a thickness of the sample at location  $(x,y)$ ;  
 $ac(x,y)$  is an intensity at location  $(x,y)$ ; and  
 $N(x,y)$  is a dimensionless number at a location  $(x,y)$ .
20. The method of claim 14, further comprising:
- (d) illuminating the sample with light; and  
15 (e) detecting light transmitted through the sample at a plurality of locations.
21. The method of claim 20, wherein the light comprises light having at least two wavelengths.
22. The method of claim 20, wherein the light comprises light having at least one wavelength in the near infrared spectrum.
- 20 23. The method of claim 14, further comprising:
- (f) displaying an image of the spatial second derivatives at the plurality of locations.
24. The method of claim 14, further comprising:
- (f) storing values of the spatial second derivatives in a computer-readable medium.
- 25 25. A method of calculating an absolute oxygenation level of a region of a tissue sample, the method comprising:
- (a) selecting a pair of wavelengths of light to minimize a difference between relative changes in intensity of light transmitted through a region of the sample, wherein

- 5 relative changes in intensity of the light are measured relative to a background intensity of light transmitted through the sample;
- (b) obtaining measures of background reduced scattering coefficients of the sample at the pair of wavelengths; and
- (c) calculating an absolute oxygenation level of the region of the sample using the
- 10 relative changes in intensity of the light at the pair of wavelengths and the measures of the background reduced scattering coefficients of the sample at the pair of wavelengths.

26. The method of claim 25, wherein the tissue sample is selected from the group consisting of breast tissue, brain tissue, and muscle tissue.

27. The method of claim 25, wherein the region of the sample comprises a lesion, tumor,
- 15 or cancerous tissue.

28. The method of claim 25, wherein the pair of wavelengths of light are in the near infrared spectrum.

29. The method of claim 25, wherein the difference between the relative changes in intensity is zero.

- 20 30. The method of claim 25, wherein the absolute oxygenation level of the region is calculated using a formula

$$SO_2 = \frac{\epsilon_{Hb}(\lambda_2) - \epsilon_{Hb}(\lambda_1) \frac{\mu'_{s0}(\lambda_1)}{\mu'_{s0}(\lambda_2)} \frac{\Delta I / I_0|_{\max}^{(\lambda_2)}}{\Delta I / I_0|_{\max}^{(\lambda_1)}}}{[\epsilon_{Hb}(\lambda_2) - \epsilon_{HbO_2}(\lambda_2)] + [\epsilon_{HbO_2}(\lambda_1) - \epsilon_{Hb}(\lambda_1)] \frac{\mu'_{s0}(\lambda_1)}{\mu'_{s0}(\lambda_2)} \frac{\Delta I / I_0|_{\max}^{(\lambda_2)}}{\Delta I / I_0|_{\max}^{(\lambda_1)}}},$$

wherein:

$SO_2$  is the oxygenation level of the region;

- 25  $\Delta I / I_0|_{\max}^{(\lambda_1)}$  is a maximum value of a relative change in intensity at a first

5 wavelength in the pair of wavelengths;

$\Delta I / I_0|_{\max}^{(\lambda_2)}$  is a maximum value of a relative change in intensity at a second wavelength in the pair of wavelengths;

$\frac{\mu'_{s0}(\lambda_1)}{\mu'_{s0}(\lambda_2)}$  is a ratio of a background reduced scattering coefficient at the first wavelength to a background reduced scattering coefficient at the second wavelength;

10  $\epsilon_{\text{Hb}}(\lambda_1)$  and  $\epsilon_{\text{Hb}}(\lambda_2)$  are molar extinction coefficients for deoxy-hemoglobin at the first and second wavelengths; and

$\epsilon_{\text{HbO}_2}(\lambda_1)$  and  $\epsilon_{\text{HbO}_2}(\lambda_2)$  are molar extinction coefficients for oxy-hemoglobin at the first and second wavelengths.

31. The method of claim 25, further comprising:

- 15 (d) illuminating the sample with a plurality of wavelengths of light; and  
(e) detecting light transmitted through the sample at a plurality of locations.

32. The method of claim 25, further comprising (d) displaying an image of the absolute oxygenation level of the region at the plurality of locations.

33. The method of claim 25, further comprising (d) storing values of the absolute oxygenation level of the region in a computer-readable medium.

34. A method of determining whether a tumor in a tissue sample is malignant or benign, the method comprising:

- 25 (a) selecting two wavelengths of light to minimize a difference between relative changes in intensity of light transmitted through the tumor for the two wavelengths, wherein the relative changes in intensity of the light for each wavelength are measured relative to a background intensity of light transmitted through the sample;

(b) obtaining measures of background reduced scattering coefficients of the sample at the two wavelengths;

5 (c) calculating an absolute oxygenation level of the tumor in the sample by using the relative changes in intensity of the light for the two wavelengths and the measures of the background reduced scattering coefficients of the sample for the two wavelengths; and

10 (d) determining whether the tumor is malignant or benign based on the value of calculated absolute oxygenation level.

35. The method of claim 34, wherein the tissue sample is selected from the group consisting of breast tissue, brain tissue, and muscle tissue.

36. The method of claim 34, wherein the two wavelengths of light are in the near infrared spectrum.

15 37. The method of claim 34, wherein the difference between the relative changes in intensity is zero.

38. The method of claim 34, wherein the absolute oxygenation level of the tumor is calculated using a formula

$$SO_2 = \frac{\varepsilon_{Hb}(\lambda_2) - \varepsilon_{Hb}(\lambda_1) \frac{\mu'_{s0}(\lambda_1)}{\mu'_{s0}(\lambda_2)} \frac{\Delta I / I_0|_{\max}^{(\lambda_2)}}{\Delta I / I_0|_{\max}^{(\lambda_1)}}}{[\varepsilon_{Hb}(\lambda_2) - \varepsilon_{HbO_2}(\lambda_2)] + [\varepsilon_{HbO_2}(\lambda_1) - \varepsilon_{Hb}(\lambda_1)] \frac{\mu'_{s0}(\lambda_1)}{\mu'_{s0}(\lambda_2)} \frac{\Delta I / I_0|_{\max}^{(\lambda_2)}}{\Delta I / I_0|_{\max}^{(\lambda_1)}}},$$

20 wherein:

$SO_2$  is the oxygenation level of the tumor;

$\Delta I / I_0|_{\max}^{(\lambda_1)}$  is a maximum value of the relative change in intensity at a first wavelength in the pair of wavelengths;

$\Delta I / I_0|_{\max}^{(\lambda_2)}$  is a maximum value of the relative change in intensity at a second

25 wavelength in the pair of wavelengths;



5  $\frac{\mu'_{s0}(\lambda_1)}{\mu'_{s0}(\lambda_2)}$  is a ratio of the background reduced scattering coefficient at the first wavelength to the background reduced scattering coefficient at the second wavelength;  
 $\epsilon_{\text{Hb}}(\lambda_1)$  and  $\epsilon_{\text{Hb}}(\lambda_2)$  are molar extinction coefficients for deoxy-hemoglobin at the first and second wavelengths; and  
 $\epsilon_{\text{HbO}_2}(\lambda_1)$  and  $\epsilon_{\text{HbO}_2}(\lambda_2)$  are molar extinction coefficients for oxy-hemoglobin at  
 10 the first and second wavelengths.

39. The method of claim 34, further comprising:

- (e) illuminating the sample with a plurality of wavelengths of light; and
- (f) detecting light transmitted through the sample at a plurality of locations.

40. The method of claim 39, further comprising (g) displaying an image of the absolute  
 15 oxygenation level at the plurality of locations within the tissue sample.

41. The method of claim 34, further comprising, (e) storing values of the absolute oxygenation level of the tumor in a computer-readable medium.

42. A method of determining whether a tumor in a tissue sample is malignant or benign, the method comprising:

- 20 (a) obtaining thicknesses of the sample and intensities of light transmitted through the sample at a plurality of locations for two wavelengths of light;
- (b) calculating spatial second derivatives of products of the sample thicknesses and the intensities of the transmitted light at the locations for the two wavelengths of light;
- 25 (c) calculating an oxygenation level of the tumor based on the spatial second derivatives for the two wavelengths of light, the molar extinction coefficients of oxy-hemoglobin for the two the wavelengths of light, the molar extinction coefficients of hemoglobin for the two wavelengths of light, relative changes in intensity of the light for the two wavelengths of light and the measures of the background reduced scattering

5 coefficients of the sample for the two wavelengths of light; and

(d) calculating an oxygenation level of non-tumor regions of the tissue sample based on the spatial second derivatives for the two wavelengths of light, the molar extinction coefficients of oxy-hemoglobin for the two wavelengths of light, the molar extinction coefficients of hemoglobin for the two wavelengths of light, relative changes  
 10 in intensity of the light for the two wavelengths of light, and the measures of the background reduced scattering coefficients of the sample for the two wavelengths of light; and

(e) comparing the oxygenation level of the tumor with the oxygenation level of non-tumor regions of the sample to determine whether the tumor is malignant or benign.

15 43. The method of claim 42, wherein the tissue sample is selected from the group consisting of breast tissue, brain tissue, and muscle tissue.

44. The method of claim 42, wherein the two wavelengths of light are in the near infrared spectrum.

45. The method of claim 42, wherein the oxygenation level of a region of the sample is  
 20 calculated using a formula

$$OL = \frac{\Delta[HbO_2]^*}{\Delta[HbO_2]^* + \Delta[Hb]^*}, \text{ wherein}$$

$$\Delta[HbO_2]^* = \frac{\left( \sum_i N''(\lambda_i) \epsilon_{HbO_2}(\lambda_i) \right) \left( \sum_i \epsilon_{Hb}^2(\lambda_i) \right) - \left( \sum_i N''(\lambda_i) \epsilon_{Hb}(\lambda_i) \right) \left( \sum_i \epsilon_{HbO_2}(\lambda_i) \epsilon_{Hb}(\lambda_i) \right)}{\left( \sum_i \epsilon_{HbO_2}^2(\lambda_i) \right) \left( \sum_i \epsilon_{Hb}^2(\lambda_i) \right) - \left( \sum_i \epsilon_{HbO_2}(\lambda_i) \epsilon_{Hb}(\lambda_i) \right)^2},$$

$$5 \quad \Delta[\text{Hb}]^* = \frac{\left( \sum_i N''(\lambda_i) \epsilon_{\text{Hb}}(\lambda_i) \right) \left( \sum_i \epsilon_{\text{HbO}_2}^2(\lambda_i) \right) - \left( \sum_i N''(\lambda_i) \epsilon_{\text{HbO}_2}(\lambda_i) \right) \left( \sum_i \epsilon_{\text{HbO}_2}(\lambda_i) \epsilon_{\text{Hb}}(\lambda_i) \right)}{\left( \sum_i \epsilon_{\text{HbO}_2}^2(\lambda_i) \right) \left( \sum_i \epsilon_{\text{Hb}}^2(\lambda_i) \right) - \left( \sum_i \epsilon_{\text{HbO}_2}(\lambda_i) \epsilon_{\text{Hb}}(\lambda_i) \right)^2}$$

and wherein:

OL is the oxygenation level of the tumor;

$i$  is a wavelength index for the two wavelengths;

10  $\epsilon_{\text{HbO}_2}$  and  $\epsilon_{\text{Hb}}$  are the molar extinction coefficients of oxy-hemoglobin and deoxy-hemoglobin, respectively;

$\Delta[\text{HbO}_2]^*$  and  $\Delta[\text{Hb}]^*$  are relative values for the spatial changes in the concentrations of oxy-hemoglobin and deoxy-hemoglobin, respectively; and

$N''$  is a spatial second derivative of an intensity of transmitted light.

15 46. The method of claim 42, further comprising:

(f) illuminating the sample with a plurality of wavelengths of light; and

(g) detecting light transmitted through the sample at a plurality of locations.

47. The method of claim 42, further comprising, (f) displaying an image of oxygenation levels at the plurality of locations within the tissue sample.

20 48. The method of claim 42, further comprising, (f) storing values of oxygenation levels at a plurality of locations within the tissue sample in a computer-readable medium.